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Some Notes on Tabanidae (Diptera) and the Description of Two New *Chrysops*

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The excellent monographic studies of Brennan (1935) and Stone (1938) on Nearctic Tabanidae and the even more recent catalog of Philip (1947) has placed the study of this group on a firm foundation. The student of this family rarely suffers from a dearth of material since the abundance of individuals of many species, their ease of preservation and their economic importance lead to the collection of long series. As these series accumulate, it sometimes is found that certain species exceed the limits of variation conceded that species, and in other cases what appear to be new forms, may be separated.

Chrysops carbonaria complex

The classification of the dark bodied group of species lacking an apical spot, which are related to *C. carbonaria* Walker, presents difficulties not noted in the literature. Many workers have suspected that *carbonaria* and *mitis* O.S. were not distinct but differences in larval characters are said to show they are different. The writer feels that further studies should be made on the immature stages of the species in this complex.

Specimens of *carbonaria* approaching *celer* O.S. have been studied by the writer. The characteristic hyaline spot at the base of the 5th posterior cell seems to show that such specimens are *carbonaria* but the yellow pleural hairs in varying amounts show a surprising resemblance to *celer*. Series of specimens of this type seem to be most common from Long Island and New Jersey localities.

Specimens determined as *carbonaria* fall rather easily into two groups although some intergrades are found. One group has the crossband with an irregular outer margin, grey hairs on the pleurae, narrow pollinose stripe separating the facial callosities and the 4th posterior cell about one third hyaline so that usually the crossband does not reach the posterior margin of the wing. This group is the one that produces individual specimens closely resembling *mitis*. The second group has a darker crossband with a straight outer margin, grey to yellow hairs on the pleurae, pollinose stripe between the facial callosities broader than in the first group—sometimes as broad as in *celer*—and the 4th posterior cell usually is wholly infuscated so that the crossband reaches the posterior margin of the wing. Specimens close to *celer* are from this second group.

A series of specimens collected by R. Lambert and H. Dietrich on July 29 and 30, 1947 at Lac Jacques Cartier, Parc des Laurentides, Quebec show interesting variations. Some of the specimens are typical *excitans* Walker but the majority are smaller *carbonaria*-like specimens with the characteristic hyaline spot in the 5th posterior cell. All of these latter specimens show varying amounts of yellow hair, such as is found in *excitans*, on the abdomen and sides and dorsum of the thorax. A few lack the hyaline spot in the 5th posterior cell but there is no correlation between its absence and the amount of yellow hairs since some of those with the greatest amount of yellow hairs have the spot and others approaching typical *carbonaria*, lack it. Two males collected at the same time seem to be the same as *carbonaria* collected elsewhere with a faint hyaline spot at the base of the 5th posterior cell.

Although the male of *C. excitans* has been reported as being completely bla[illegible]e writer has studied a specimen from Midland County, Michigan, 6 June 1[illegible]R. R. Dreisbach) which resembles the female in having yellowish lateral [illegible] on the first two abdominal segments and thick yellow pleural hairs. It shows yellow median markings on the abdomen but such spots are often lacking on [illegible]males collected in Michigan. The dorsum of the thorax has more black than yellow hairs while in the female the reverse is true.

At this time the writer does not pretend to have a solution for the taxonomic problems raised but he feels that we should not rule out the possibility of occasional hybridization in this group.

Chrysops callida complex

Specimens of the group of species related to *C. callida* O.S. from inland localities are usually easily placed although occasional specimens of *C. aestuans* v.d. Wulp are close to *callida*. However, long series collected in recent years from Long Island and New Jersey localities are difficult to separate. The only structural character which seems to have value is the shape of the frontal callosity and that character breaks down in some series. The usual differentiating characters of wing and abdominal pattern and leg coloration hold only fairly well when long series are studied. The writer suggests that considerable study will be necessary to determine if *C. callida* O.S., *C. pudica* O.S., *C. sackeni* Hine, *C. dimmocki* Hine and *C. cursim* Whitn. are all valid species.

One member of the *callida* complex, *C. delicatula* O.S. seems to be quite distinct. Although it is somewhat variable, the writer has seen no specimens which would be confused with other species of the *callida* complex. The hyaline basal portion of the discal cell seems quite constant, but the black markings of the abdomen vary considerably in extent and the dark spots on the frontoclypeus, which are black and extensive in most specimens, are reduced to faint shadows in a few specimens. The black area of the genae varies in extent but has always been present in the specimens examined.

The male of *C. delicatula* seems to be unknown. The writer has studied a single male collected 3 August 1947 at Calverton (L.I.), New York (Roy Latham). It is easily associated with the female with essentially the same abdominal pattern although the background color is brighter yellow than in most females. The wing pattern is similar to that of the female but is darker, the apical spot is slightly broader towards its apex, the 5th posterior cell and apex of the anal cell are more heavily infuscated and the basal cells are about two thirds infuscated basally and, in addition, the 1st basal cell is infuscated at its apex. The characteristic hyaline area at the base of the discal cell is clearly present but somewhat smaller than in most females. Legs and antennae are colored as in the female. The black spots on the frontoclypeus and genae are larger than in most females and the yellow area is reduced to a narrow stripe in the centre of the frontoclypeus.

The male of *C. delicatula* keys to couplet 17 in Brennan's key (1935, p. 260) which includes two unrelated species, *surda* O.S. and *proclivis* O.S. It is separated from these species by the dark legs, narrower apical spot, straight outer margin of the crossband, and entirely different abdominal pattern. It is separated from males of the *callida* complex by the dark spots on the frontoclypeus and genae. The hyaline area at the base of the discal cell also is characteristic but occasional specimens of *cursim* show a similar condition.

Chrysops furcata Walker

Specimens of this species from Quebec and New Brunswick are somewhat different from western specimens. They are smaller and darker and have a lateral spot on each side of the median spot on the 2nd abdominal tergite which some-

times is large enough to touch the median figure. On the 3rd, 4th and 5th tergites the yello[w] is reduced to narrow posterior bands which expand to form a [tri]angular triangle which does not reach the anterior margin. A single female from [N]ewfoundland is very small and has the yellow of the abdomen even further redu[ced]. These specimens probably represent an eastern subspecies of *C. furcata* althou[gh] some intermediate characters are shown by a series of females from Alberta.

***Chrysops montana* Osten Sacken**

One of the key characters used by Brennan (1935, p. 259) and others to separate *C. montana* from related species, is the presence of a black spot on each side of the median figure on the second abdominal segment. Many specimens of this species studied by the writer lack these spots and in others they are so reduced as to be nearly invisible. The absence of these spots is especially common in specimens from along the Atlantic coast but specimens from other areas occasionally lack them. *C. montana* appears somewhat variable also in the extent of other dark abdominal markings and in the extent of the apical spot of the wing.

***Chrysops flavida* complex**

The *Chrysops flavida* complex of species is a confusing one and the writer at this time is not making an attempt to clarify it completely. However, in the past few years much material of this complex has accumulated and in this are two forms, the recognition of which should help in future work on the group.

***Chrysops atlantica* n. sp.**

Holotype Female. Length 9 mm.

Head: Antennae deep yellow except flagellum which is dark brown; scape moderately swollen, pedicel somewhat less so; scape and pedicel densely covered with stiff black hairs; pedicel slightly shorter than scape and 3rd segment slightly longer than total length of first two segments. Front slightly higher than wide, somewhat narrowed above, pale yellow pollinose and thinly covered with golden hairs; area between ocelli dark brown; frontal callosity yellow, large, oval and narrowly separated from eyes; a narrow, deep, pollinose depression runs from lower margin of frontal callosity between the antennae. Frontoclypeus shining yellow; a narrow pollinose band runs along margin of eye and enlarges somewhat below. Palpi deep yellow with black hairs. Proboscis yellow-brown, shading to dark brown below.

Thorax: Dorsum greyish-green in ground color with the usual three blackish stripes; centre stripe much narrower than outer stripes. Pleurae similar in ground color to dorsum with a few yellow pollinose areas and a brown stripe anterior of wing bases. Disc of scutellum concolorous with dorsum; sides and tip, yellow-brown. Legs mostly yellow; hind coxae and most of tarsi fuscous; front tibiae deeper in color than other tibiae and with more black hairs; middle and hind tibiae with mixture of yellow and black hairs. Halteres yellow-brown, somewhat lighter on the knob. Wing with a smoky tinge which is only slightly paler in the 2nd basal cell and posterior portion of the anal cell; the margins of the usual wing markings are not clearly defined since they have a tendency to fade into the smoky background of the wing; outer margin of the crossband somewhat irregular, crossing 1st posterior cell near its centre, cutting off extreme base of 2nd posterior cell and basal third of 3rd posterior cell; all of the 4th and 5th posterior cells are included in the crossband but the central portion of the 5th posterior cell is paler in color; apical spot broadly crosses the upper branch of the 3rd longitudinal vein and then fades into the background color of the wing in the 2nd submarginal cell; hyaline triangle does not reach the 2nd longitudinal vein; costal, subcostal and marginal cells infuscated, slightly less than half of basal

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portion of 1st basal cell and extreme base of 2nd basal cell infuscated; anal cell infuscated where it adjoins 5th posterior cell.

Abdomen: General ground color brownish-yellow; 1st tergite with a trace of darker marking beneath scutellum; 2nd tergite with a geminate black spot, the two portions of which are narrowly in contact anteriorly and which does not touch any margin of the segment; ground color of segment somewhat paler below geminate spot giving the impression of a pale triangle in some lights; 3rd and 4th tergites similar to 2nd but geminate spot is smaller and separated into two spots which touch the anterior margins of the segment; 5th tergite with four black spots along anterior margin of segment and the 6th tergite has an indistinct black band along the anterior margin; dorsum of abdomen with a mixture of fine golden and black hairs with the golden hairs becoming quite long on the posterior margins of the 4th and following segments. Sternites yellowish, the 4th and following segments somewhat dusky along the posterior margins.

Rehoboth Beach, Delaware, 6 July 1938.

Allotype Male. Length 8.5 mm.

Much like female holotype except for usual sexual differences.

Head: Color and relative proportions of antennal segments same as female; hair on first two segments longer and finer than in female and scape somewhat more swollen. Frontoclypeus shining yellow; distribution of yellow pollinose areas similar to female. Palpi yellow-brown with long black hair. Proboscis yellow-brown.

Thorax: Similar to female but with dorsal stripes less obvious. Scutellum concolorous with thorax, only extreme tip showing a tinge of brown. Legs and halteres as in female. Wings as in female but with outline of crossband and apical spot even more indistinct.

Abdomen: General ground color a deeper shade of brownish yellow than in female; geminate spot on 2nd tergite appears as a single crescent shaped spot not touching any margin; 3rd and 4th tergites as in female but spots not quite touching anterior margin; 5th and 6th tergites largely black with narrow pale margin which is slightly expanded on the median line of the 5th tergite; mixture of yellow and black hairs on abdomen somewhat longer than in female. Venter yellowish, a faint dark median spot on the 2nd, 3rd and 4th sternites; posterior sternites mostly blackish.

Odessa, Delaware, 4 June 1936 (Donald MacCreary).

Paratypes: A long series of *C. atlantica* was studied and paratypes selected from the following localities. All are females unless otherwise noted. *Massachusetts*: Kent's Island, Newbury, 17 July 1947 (N. S. Bailey). Pine Island, Newbury, 25, 26 July 1946; 8, 9, 21, 24 July 1947; 7, 13 August 1947 (N. S. Bailey). Gloucester, 11 July 1947 (N. S. Bailey). Sagamore, "biting man" (J. Bequaert). Barnstable Dunes (K. Cooper); Barnstable Harbor, 26 July 1939; 21 August 1939 (R. L. Armstrong). Parker River Marsh, W. Yarmouth, 17 August 1939 (R. L. Armstrong). Dennis, 31 August 1917 (A. P. Morse). Woods Hole, 12 July (R. C. Osburn). Tisbury, 7 July 1934. Martha's Vineyard, 23 July 1930. *Rhode Island*: Westerly, 18 July 1935 ♂ (M. Chapman). *Connecticut*: Lyme, 24 June 1918; 23 July 1918 (W. S. Fisher). Lyme, 29 June 1918 (C. T. Greene). *New York* (Staten and Long Island): Orient Pt., 10 July 1910. Orient, 30 June 1948; 16, 21, 30 July 1948; 2, 3, 4, 7, 9, 10, 11, 12, 13, 14, 15, 18, 20 August 1948; 1, 4, 6, 7, 8, 10, 11, 13, 16, 17, 24, 25, 27, 30 September 1948; 4 October 1947 (Roy Latham). Greenport, 2 August 1941; 1 October 1947 (Roy Latham). Napeague, 8 May 1946 (Roy Latham). Sag Harbor, 17 April 1947 (Roy Latham). Flanders, 6 October 1947 (Roy Latham). Riverhead, 30 June

1921. Riverhead, 1 August 1947 (Roy Latham). Calverton, 4 July 1941 (Roy Latham). Rosedale, 30 June 1918 (F. M. Schott). New Dorp, 1 July 1925 (W. T. Davis). *New Jersey* (all collected by E. J. Hansens unless otherwise indicated): "N.J.", 19 July 1891 (J. M. Aldrich). Island Beach, 9 July 1947; 5, 11 August 1947; 28 June 1948. Lanings Wharf, 11 July 1947. Metedeconk, 9 July 1947. Cedar Run, 25 June 1947. West Creek, 6 July 1935 (K. V. Krombein). Tuckerton, 23 June 1947; 29 June 1948; 9, 16, 31 July 1947. Tuckerton, 5 August 1947 (G. W. Barber). New Gretna, 20 June 1947. Leeds Point, 29 June 1948. Swainton, 27 June 1947. Avalon, 29 June 1948. Cape May Court House, 23 July 1946 (J. N. Belkin). Cape May, 2 July 1916 (F. Haimbach). Cape May, 5 July 1933 ♂ (Witmer Stone); 24 June 1933; 15, 30 August 1933; 19 August 1938 (Witmer Stone). Dias Creek, 24 June 1948; Goshen, 26 June 1947. Leesburg, 27 June 1947. Port Norris, 15 June 1948. Dividing Creek, 16 June 1948; 4, 17 July 1947; 22 July 1948. Fortesque, 4 July 1947. Sea Breeze, 11 June 1947. *Delaware* (all collected by Donald MacCreary unless otherwise indicated): Oyster Rock, 23 June 1938. Delaware City, 26 June 1934. Odessa, 1, 4, 9, 12 June 1936; 8, 21 June 1937. Leipsic, 26, 28 May 1937 ♂ (reared); 25, 30 June 1938 ♂ ♀; 2, 9 July 1937; 18 August 1938. Dover, 27 June 1937; 29, 30 June 1939; 25, 29 August 1938; 5 September 1938 (R. Traub). Magnolia, Kent Co., 16 July 1938 (Rehn and Rehn). Indian River Bay, 11 August 1936. Flemings Landing, 8 June 1937. Dora Landing, 8 June 1934, 13 June 1935. Nassau, 7 July 1937. Rehoboth, 29 August 1938 (R. Traub). Rehoboth Beach, 30 June 1938; 6, 14 July 1938; 16 July 1937; 18 August 1938. Rehoboth Bay, 14 June 1937; 2, 15, 29 July 1937; 14 July 1938; 11, 17 August 1937. *Maryland*: Dorchester Co., near Lloyds, 10 July 1907 (H. S. Barber). Cambridge, 29 May 1939 (H. L. Dozier). Idlewild, 5 July 1920 (A. N. Caudell). *Virginia*: Deep Creek, 7 June (H. S. Barber).

Holotype and Allotype in writer's collection. Paratypes in United States National Museum, Canadian National Collection, British Museum (Natural History), Museum of Comparative Zoology, Cornell University, Ontario Agricultural College, Rutgers University, University of California, California Academy of Sciences, and in the collection of C. B. Philip.

Variations: In some females the pollen of the front has a greenish cast and in some the antennae are a deep yellowish brown. The scutellum is entirely concolorous with the dorsum of the thorax in some specimens and others show more yellow brown than the holotype. In some specimens the smoky tinge of the wing almost completely obscures the usual wing pattern.

Considerable melanism is shown by a number of specimens of both sexes. This varies from slight enlargement of the abdominal spots to an extreme in which the yellow-brown background of the abdomen is reduced to a narrow posterior band on the first tergite and, on the 2nd and following tergites, to a narrow posterior band with a triangular enlargement on the median line. When the abdomen shows intensified markings, the sternites often have a median dark spot and the posterior sternites show dark posterior borders. Specimens showing extreme melanism are not included in the paratype series. A few specimens have the dark abdominal markings somewhat reduced when compared with the holotype.

Female paratypes vary in length from 6.5 mm. to 9.5 mm. but most specimens are close to 9 mm; one specimen, not included in the series of paratypes is 5.5 mm. Male paratypes vary in length from 7 mm. to 9 mm.

The species which the writer has called *C. atlantica* has been known for some time but has been confused with *flavida* Wied. and *brunnea* Hine. Krober

(1926, p. 291) apparently had both *atlantica* and *flavida* before him when he redescribed *flavida*. Philip (1941, p. 118) refers under *flavida* to "a northern form which approaches *brunnea* Hine". Daecke (1907, p. 146) mentions specimens of *flavida*, "in a measure resembling *brunneus*" and his figure 33A on plate VI is an excellent delineation of the wing of *atlantica*. MacCreary (1940, p. 11) mentions an anomalous form of *flavida* being more common than typical *flavida* in Delaware.

C. atlantica differs from *brunnea* in that *brunnea* has the first two antennal segments more swollen, the abdomen is a uniform brown with markings, if any, showing only as dark shadows and the thorax usually is predominantly brownish. The frontal callosity in *brunnea* usually is more protuberant and the wing pattern is a deeper color with the hyaline areas of the membrane lacking the yellowish tinge found in *atlantica*.

C. atlantica differs from *flavida* and its subspecies in that *flavida* usually has the first two antennal segments more slender, the abdomen pale yellowish brown with faint brown markings and the wing pattern distinctly outlined. However, specimens included in what is called *flavida* show great variation and seem to form local races. Along the coast of the Carolinas, Georgia and Florida, specimens with infuscated wing membranes are found but are separated from *atlantica* by the brown thorax and different body coloration which often closely resembles *brunnea*. The writer also has seen long series of the *flavida* complex from southern Florida which have antennae much like *brunnea* but with wing and body pattern close to *flavida*.

The darker specimens of *atlantica* have abdominal markings which somewhat resemble those of *cursim* and *pudica* but the clear cut wing pattern and brighter yellow abdominal coloration of these species is distinctive. Certain specimens, of what is probably *pudica* or a closely related form, occasionally show some tinting of the wing membrane but the crossband and apical spot are darker than in *atlantica* and they are smaller. Some of these latter specimens are quite melanistic. Fairchild (1937, p. 60) mentions the possibility of several species being included in Florida specimens of what is called *pudica*.

Chrysops flavida subspecies _celata_ n. subsp.

Holotype Female. Length 8.25 mm.

Head: Antennae dark yellow, darker at base of 3rd segment; flagellum black; scape and pedicel slightly swollen and with stiff black hairs; pedicel about two thirds length of scape; 3rd segment slightly longer than total length of 1st two segments. Front greenish yellow pollinose with scattered yellow hairs; frontal callosity yellow with a narrow black border above, large and narrowly separated from eyes. Frontoclypeus shining yellow; a narrow yellow pollinose band runs along margin of eyes and widens somewhat below and opposite frontoclypeal pits. Palpi dark yellow with black hairs. Proboscis yellow-brown; labellae dark brown.

Thorax: Dorsum greyish-green in ground color with three fuscous stripes; centre stripe narrower than outer stripes; dorsum with a yellowish tinge above wing bases. Pleurae similar in ground color to dorsum with some greenish yellow areas. Scutellum concolorous with thorax with a trace of reddish brown at apex. Legs yellow, apical portion of hind tibiae, hind coxae and trochanters yellow-brown; apical third of fore tibiae and fore tarsi fuscous and other tarsi fuscous at apex. Halteres brown. Wing picture distinct; outer margin of crossband somewhat sinuate, beginning just below 2nd longitudinal vein, crossing the 1st posterior cell near its centre and cutting off the extreme base of the 2nd posterior cell and basal third of the 3rd posterior cell; all of the 4th and 5th posterior cells

and apical portion of the anal cell are included in the crossband but the 5th posterior cell is somewhat lighter in color in the centre and apical portions; costal, subcostal and marginal cells are infuscated as is basal third and extreme apex of the 1st basal cell and extreme base of 2nd basal cell; apical spot broadly crosses about seven-eighths of the upper branch of the 3rd longitudinal vein and occupies about half of the 2nd submarginal cell.

Abdomen: General ground color dull yellowish; 2nd segment with a pale brown geminate spot which reaches the posterior margin but not quite reaches the anterior margin; 3rd and following segments with a pale brown marking which is bordered along the posterior margin of each segment by the ground color which expands into a triangle on the median line of the segment but which does not reach the anterior margin; the brown markings are covered with dark hairs and the yellow ground color is covered with yellow hairs. Sternites yellow; 5th and following sternites fuscous along anterior margins.

Medford Lakes, New Jersey, 20 June 1935 (L. L. Pechuman).

Paratypes: *New York*: Babylon, 28 June 1937; 1, 2, 6, 9 July 1937 (Blanton & Borders). Cold Spring Harbor, 29 June 1931 (C. H. Curran). Yaphank, 6 July 1936 (W. G. Bodenstein). Riverhead, 30 June 1921. *New Jersey*: Lahaway, 25 July 1946 (J. N. Belkin). Colliers Mills, 25 July 1946 (J. N. Belkin). Cape May, 4 August 1917 (Witmer Stone). Lake Denmark, 21 July 1948 (D. M. Jobbins). Clayton, 11 July 1947 (E. J. Hansens). Moores Beach, 25 June 1948 (E. J. Hansens). Manahawkin, 21 June 1935 (L. L. Pechuman). *Alabama*: Pryor Springs, Decatur, 27 May 1941; 9, 11 June 1941 (J. N. Belkin).

Other specimens not included in the series of paratypes are from Delaware, North Carolina, South Carolina and Tennessee.

Holotype in writer's collection. Paratypes in United States National Museum, Canadian National Collection, British Museum (Natural History), Museum of Comparative Zoology, Cornell University, Rutgers University and in the collection of C. B. Philip.

Variations: Specimens vary in length from 8 mm. to 9 mm. The color of the scutellum varies from completely concolorous with the thorax to having the apical three quarters reddish brown. A few specimens have the base of the hind femora somewhat darkened and a few have the abdominal spots intensified so that they are dark brown instead of pale brown as in the type.

Chrysops flavida subsp. *celata* is most easily separated from *C. flavida* and from *C. flavida* subsp. *reicherti* Fairchild by the dark thoracic dorsum with dark stripes. The sinuate or convex outer margin of the crossband is fairly distinctive although this type of margin is rarely found in typical *flavida* and is usual in *reicherti*. The exact status of *C. flavida* subsp. *celata* is difficult to determine. It appears to be found throughout most of the range of *flavida* and probably is not a true subspecies in the strict sense. It shows some characters of the *callida* complex, especially such forms as *C. dimmocki*.

***Tabanus fuscicostatus* Hine**

A series of both sexes of this species collected at Kissimmee Prairie, Florida, 25 August 1931 (J. G. Needham) seems to include the first males of this species of which there is any record. The males key out to couplet 53 in Stone's key (1938, p. 50).

The males differ from those of *T. nigrovittatus* Macq. in having a darker colored costal cell. The specimens at hand all differ from male *nigrovittatus* seen by me, collected at various localities along the Atlantic coast, in the narrower basal portion of the 3rd antennal segment, by the annulate portion of the 3rd antennal segment being about the same length as the basal portion, by the upper facets of the eye being much larger than those in the lower portion, whereas in

nigrovittatus the difference in size is less, and by the absence of dark median markings on the sternites.

From *T. mularis* Stone the males of *fuscicostatus* are differentiated by the much paler abdomen and from *T. quinquevittatus* Wied. they are separated by the pale pleural hairs and white palpi.

The length of the males in the series is from 9 mm. to 10.5 mm.

***Tabanus birdiei* Whitney**

A single male of this species collected at Lake Placid, Florida, 20 March 1945 (J. G. Needham) seems to be the first of that sex to be reported.

The female of *T. birdiei* bears a superficial resemblance to *T. cymatophorus* O.S. but is easily distinguished by characters of front, antennae and legs. Unfortunately the characters of the front are absent in the male and those of the legs are not quite as distinct as in the female.

The male of *T. birdiei* goes to *cymatophorus* in couplet 35 of Stone's key (1938, p. 49) but differs from that species in having the 3rd antennal segment dark except at the extreme base and the fore tibiae somewhat paler at the base but not markedly bicolored as in *cymatophorus*. The length of the specimen is 16 mm.

***Tabanus* (*Hybomitra*) *criddlei* Brooks**

A number of years ago the writer examined the specimens in the Canadian National Collection later used by Brooks in describing *T. criddlei*. The presence of specimens from Algonquin Park which showed some intergrading characters with *T. cinctus* Fab., seemed to indicate that the relationship of this form could best be expressed by calling it a subspecies of *T. cinctus*.

Additional material of *T. criddlei* from the collection of the Ontario Agricultural College was examined recently by the writer. Two males and two females are from the type locality, Aweme, Manitoba, collected by N. Criddle in various years on June 25 and 30 (males) and July 6 (females). Two males are from Algonquin Park, Ontario, 19 June 1932 (J. McDunnough) and show the same intergrading characters with *T. cinctus* noted above in the specimens in the Canadian National Collection. Female specimens from Algonquin Park should help in determining the true nomenclatural status of *T. criddlei*.

***Tabanus lacustris* Ghidini**

Ghidini (1938, p. 343) proposed the above name for a species from Abyssinia but it is preoccupied by *Tabanus lacustris* Stone (1935, p. 13). The writer advised Prof. Ghidini of this in 1939 but has been unable to find that a new name has been suggested. The writer proposes that this species be named *Tabanus ghidini*.

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Laboratory Technique for Testing Codling Moth Insecticides*

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In 1944, as soon as DDT became available, laboratory investigation of its possibilities for control of codling moth, *Carpocapsa pomonella* (L.) was started at Vineland Station. The work was later expanded and the methods improved until the present technique was evolved; this is still unsatisfactory in many

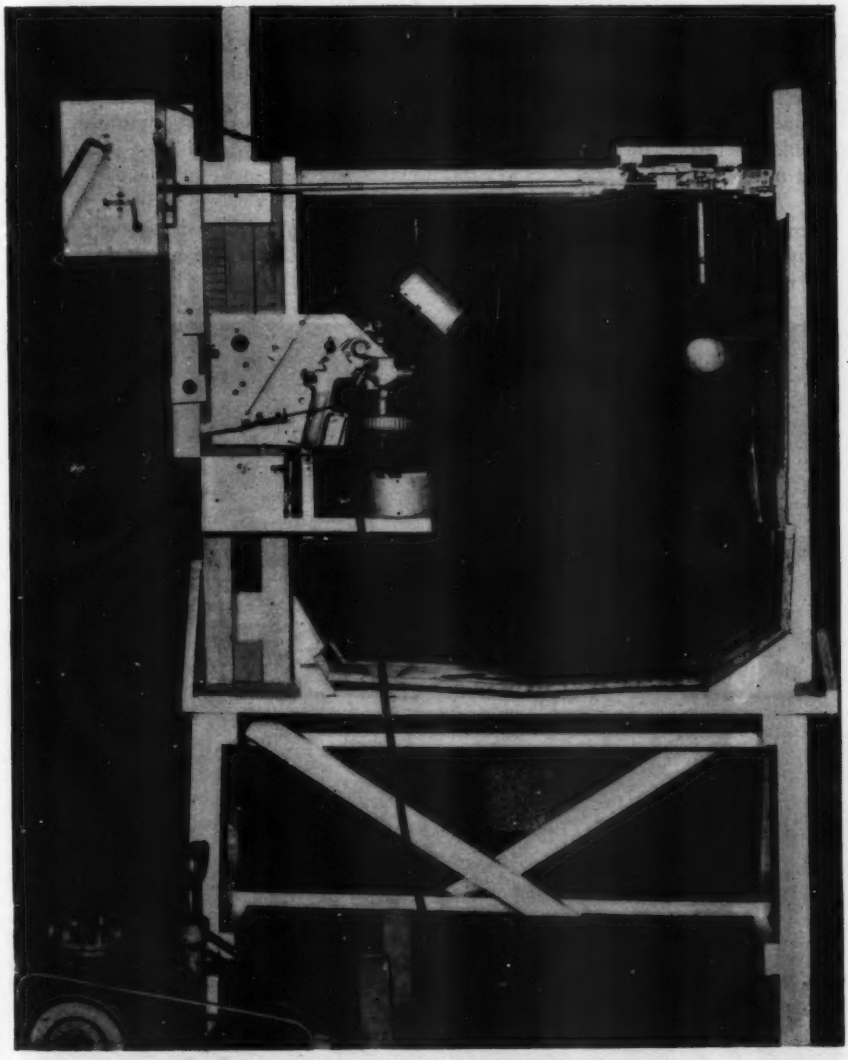

FIG. 1. Laboratory Sprayer for Testing Codling Moth Insecticides.

*Contribution No. 2609, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

respects but is presented to indicate what may be accomplished in a small laboratory with very limited personnel and equipment and at comparatively little cost.

Little originality is claimed for either equipment or methods. Ideas have been gleaned consciously or unconsciously from many sources, to which proper credit cannot now be given, but the papers edited by Campbell and Moulton (1943) have been of great assistance. Various members of the staff of this laboratory have also made valuable suggestions.

The Sprayer

The sprayer, shown in Fig. 1, was essentially a device for rotating the apples at constant speed for a definite period in the discharge of the spray nozzle. As new metal parts could not be obtained during the war, it was built entirely from scrap, including discarded radio parts, old typewriters, and phonographs. It would therefore be difficult to reproduce and precise details and dimensions will not be given.

A wooden base, 24 inches high, bore at one end two uprights. The fruit-rotating mechanism formed a unit, and the spray nozzle and associated parts comprised another unit, each capable of independent vertical adjustment along the uprights.

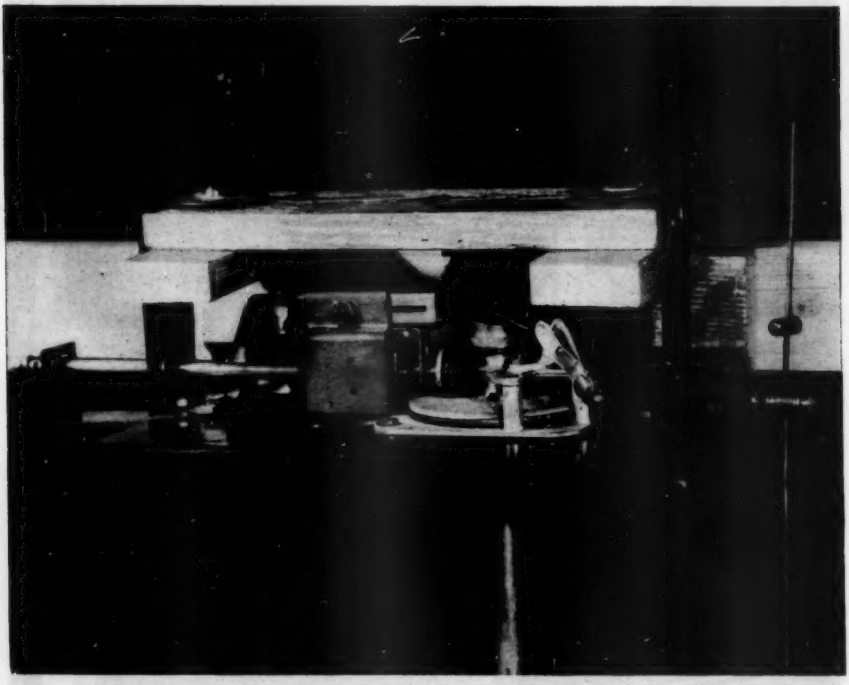

FIG. 2. Revolving Head of Laboratory Sprayer.

The rotating unit was a horizontal wooden bar bearing at one end a phonograph motor connected by a shaft with the revolving head near the other end. The shaft was made from the telescoping leg of a camera tripod, thus allowing

the revolving head to be moved along the bar so that the distance between the fruit and the spray nozzle could be varied.

In the revolving head (Fig. 2) the horizontal shaft drove a vertical spindle through a variable-speed friction drive, a small fibre disc on the end of the shaft bearing upon a larger, horizontal, leather-covered disc attached to the upper end of the spindle. The speed of the latter could be adjusted by altering the position of the smaller disc upon the larger one, and finer adjustment was made by means of the speed control on the motor. It was found that 30 revolutions per minute was satisfactory, a higher rate tending to throw the spray off

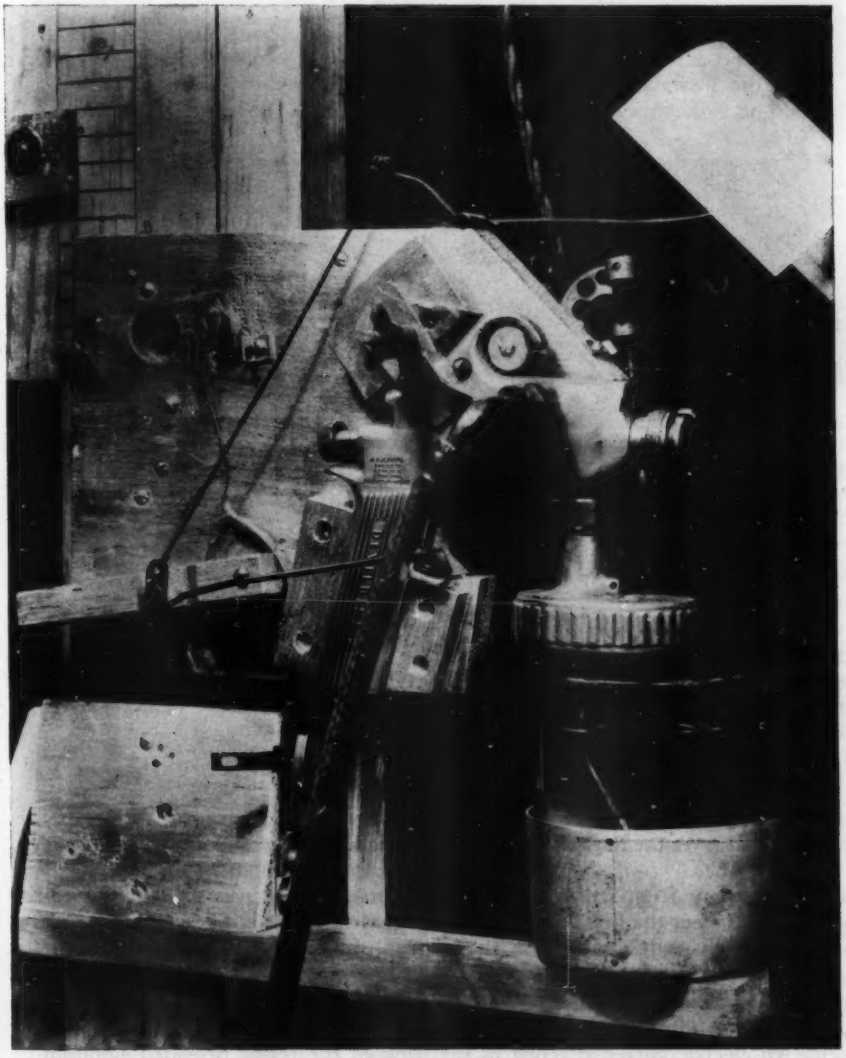

FIG. 3. Nozzle Mechanism of Laboratory Sprayer.

the fruit by centrifugal force. At the lower end of the spindle was a hook, on which the apple was hung. The spindle itself consisted of two telescoping tubes; its length could be quickly adjusted by loosening a thumb screw so that the apple would hang on a level with a wire that indicated the centre of the spray discharge. The hook turned freely in the spindle so that it could be stopped with the fingers while the fruits were being changed. If the complete rotating mechanism was stopped, by a spring brake bearing on the shaft, too much time was lost while the motor picked up speed.

A cam on the upper end of the spindle above the driving disc raised the hammer of a bell and let it fall to signal each revolution of the fruit. An auditory signal was found easier to follow than a visual one.

The spray was discharged through a DeVilbiss paint gun, held in a fixed position on a frame by a spring clamp (Fig. 3). When the gun was not operating, the materials settled out of suspension in the intake tube, or unstable emulsions sometimes partially broke down within the gun. To prevent this

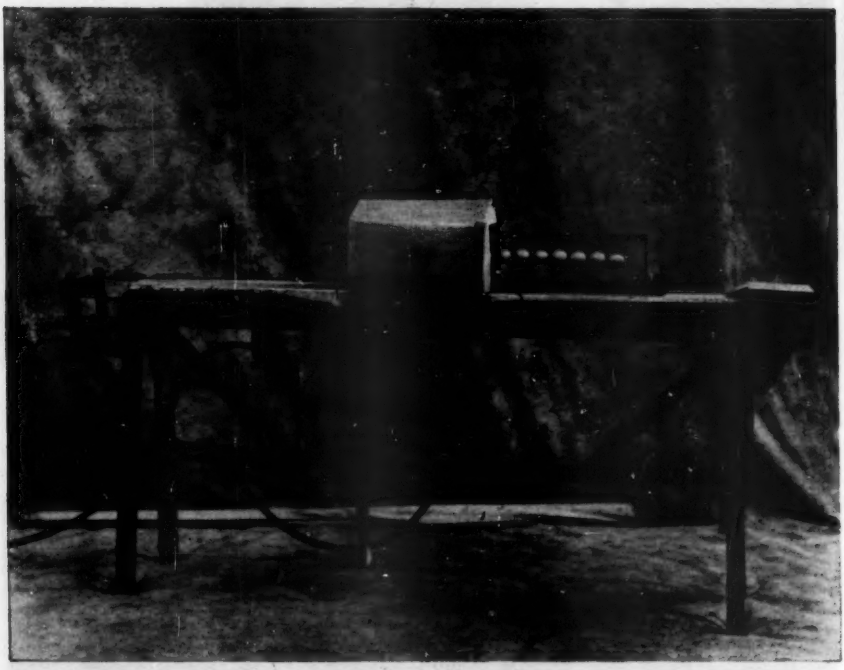

FIG. 4. Washer for Testing Weather Resistance of Spray Deposits on Apples.

stagnant spray from striking the fruit, a curved shield was pivoted before the nozzle to deflect the first discharge of the gun downward. The control lever was connected by wires with both the trigger of the gun and the shield; partial depression of the lever compressed the trigger and started the discharge of the spray against the shield. After about three seconds, the lever was completely depressed, raising the shield and allowing the spray to hit the fruit.

An electric stirrer attached to the back of the frame provided agitation of the spray mixture.

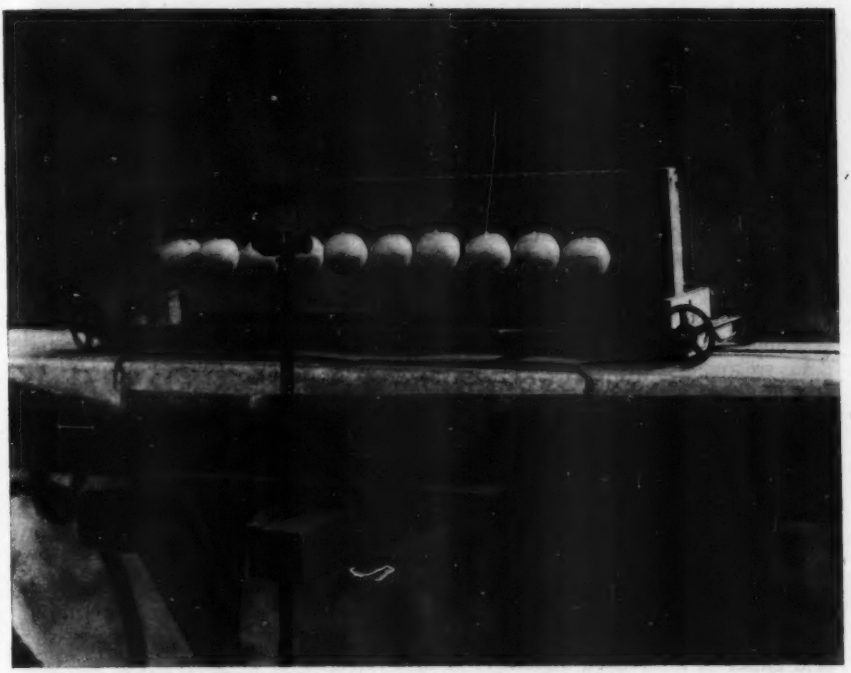

FIG. 5. Washer with Hood Removed.

The spray reservoir was a 600 ml. beaker, held in a support which slid along the uprights so that it could be lowered to change beakers or raised to latch in a fixed position against the gun frame with the sprayer intake and the stirrer propellor dipping into the spray liquid.

Air pressure was provided by a portable electric compressor designed to operate the gun as a paint sprayer. A regulator and a gauge in the air line allowed adjustment of the pressure, which was usually kept at 20 pounds.

As the level of liquid in the beaker fell during spraying, the rate of discharge, and presumably the deposit on the fruit, decreased slightly. To overcome this difficulty as far as possible, a fresh beaker containing 500 ml. of spray mixture was always inserted for each test lot of ten fruits; thus each test as a whole received the same amount of spray despite the slight variation among individual fruits.

The Washer

To determine the resistance of the spray deposits to weathering, the fruits were passed through a washer (Figs. 4 and 5), in which they were exposed to a spray of water to simulate the effect of rain.

A wooden bench seven feet long and eight inches wide was covered with galvanized iron, with the edges bent down at the sides to form gutters leading to a spout which conducted the water into the basement drain. At the middle of the bench, two nozzles from discarded knapsack sprayers directed their spray inward from opposite sides. They were connected by hose with the laboratory water supply; a pressure regulator and gauge allowed the pressure to be held constant at sixteen pounds. A hood of galvanized iron intercepted any spray passing beyond the fruits.

A phonograph motor was attached to a shelf at one end of the bench. An endless belt made of stout cord passed around the spring drum of the motor, along the top of the bench to a pulley at the opposite end, and back beneath the bench of the drum. The pulley was attached to a pivoted bracket with a weight to keep the belt taut.

The apples were washed while still on the racks, on each of which were hung, after being sprayed, one complete test lot of ten fruits. Each rack was placed on a four-wheeled trolley which was clipped on to the belt and pulled along the bench through the discharge of the nozzles at the rate of six inches per minute. Two trolleys were used so that one rack could be started along the bench while another was still between the nozzles.

As the fruits were not revolved, all surfaces did not receive equally thorough washing, but this detail was not important because all the apples received the same treatment and the amount of washing was purely arbitrary. No attempt was made to calibrate the washer in terms of inches of rainfall, as the effect of rain on spray deposits is doubtless influenced by other factors such as intensity, size of drops, and driving by wind.

Production of Larvae

Mature codling moth larvae were collected in late summer and autumn from corrugated paper or burlap tree-bands and were allowed to respin their cocoons in corrugated paper rolls. Collection of larvae has recently become very difficult because most orchards now have low infestations as a result of DDT spraying. The local collections were sometimes supplemented by others made through the co-operation of other Dominion Fruit Insect laboratories. The larvae were held either in a screened insectary, or in a refrigerator at 35-37°F; in the latter they were kept in a closed container to prevent desiccation. Beginning about the middle of February, lots of larvae were placed in glass jars in an incubator at 80°F. and high relative humidity. Larvae incubated before this time transformed irregularly and egg production from the moths was very poor. There was no appreciable difference in the rates of emergence of adults from larvae held in the insectary and from those held in the refrigerator when they were not incubated before the middle of February. After the moths had begun to emerge, production was maintained at the required level by transferring the jars back and forth between the incubator and the refrigerator.

Oviposition cages of various types were constructed, but that found to give the greatest egg production per female with a reasonable expenditure of time was a modification of one originally designed by H. Andison at the Dominion Entomological Laboratory, Summerland, B.C. A wooden base, nine inches square, was covered by a piece of glass the same size, and the glass covered with waxed paper. A cylinder of 16-mesh galvanized wire screen eight inches in diameter and 11 inches high was set upon the paper; another piece of waxed paper was put on top of the cylinder, and finally another square of glass. A piece of wire with hooked ends was placed across the upper glass and held in place by rubber bands stretched from the hook on this wire to others on two sides of the wood base. A coil of dental cotton saturated with water was placed in the centre of the lower paper. About 75 moths were usually put in the cage. When the cage was examined, the top glass and paper were removed and the dental cotton was taken out. A fresh piece of paper was put over the top and the glass replaced, and the whole cage, except the wooden base, was then inverted upon the latter. A new coil of wet cotton was placed inside and the upper (formerly the lower) paper changed. Sufficient newly emerged moths were added daily to replace those dying, and the latter were removed every three or four days. Most of the

eggs were laid on the lower paper near the cotton. The cages were kept in an incubator at 80°F. and 85-90 per cent relative humidity.

Erratic egg production gave much trouble throughout the tests. Moths from larvae obtained from different localities, even from orchards a few miles apart, differed greatly in this respect. A considerable range of temperature, humidity, and intensity and periodicity of light, was tried within the limits of the equipment, but conditions which appeared to give best results at one time might not do so a few weeks later. A very faint continuous light gave as good results as any other form of lighting. A can of over-ripe apples in the incubator sometimes appeared to stimulate oviposition. Sudden changes in the weather, especially a warm spell, often led to increased production although the cages were kept in the incubator at constant temperature; possibly changes in barometric pressure were involved. A cage which once began to produce many eggs usually continued to do so until the moths died. This fact, and the deposition of many infertile eggs, suggested that lack of mating was the chief cause of poor egg production.

The waxed paper was cut into small triangular pieces bearing single eggs or groups and placed on pins to give a total of 10 fertile eggs per pin. This operation could be done most easily when the eggs were in the 'black-spot' stage, shortly before hatching; but as at this stage they were very susceptible to the dry atmosphere of the laboratory it was necessary to prepare them the day after they were laid. They were always kept in closed jars with moist blotting paper in the bottom.

The eggs were placed on the fruit when the oldest were in the 'black-spot' stage and due to hatch within a few hours, but as each day's eggs comprised all those laid over a 24-hour period, they continued to hatch for a similar length of time. By holding them for different periods in two incubators running at 70 and 80°F., respectively, or up to two days in the refrigerator, eggs laid over several days could be brought to a uniform stage of incubation for a single day's tests. There was no detectable difference in the vitality of the eggs held at the different temperatures, but care was taken to distribute any one day's eggs equally among all the tests run at one time.

Preparation and Spraying of Fruit

Wealthy apples were picked about the first of August and held in commercial cold storage until needed. This proved to be the best of the few varieties locally available in sufficient quantities, but the fruit usually began to break down rapidly when taken out of storage after early May. It is probable that some other varieties would have been better; a few Jonathans which were used one season remained in good condition very much longer.

The fruits were washed in three per cent hydrochloric acid to remove lead arsenate residue. It was necessary to dip the Jonathans in acetone for two seconds to remove the epidermal grease, which was toxic to the larvae and reduced the rate of establishment. The acetone bath did not consistently increase larval establishment in Wealthy and was omitted in most tests.

The stems were removed, and wire hooks made from paper clips were inserted into the fruits. The cavities at both stem and calyx ends were filled flush with melted paraffin, sufficiently hot to make a smooth, tight union with the epidermis and not to leave a raised margin under which the larvae might enter. Immediately after being waxed, the apples were placed in closed cans in the refrigerator until required.

Before being sprayed, the apples were hung on racks, each holding ten fruits which received the same spray and subsequent treatment. After they had

approximated the temperature of the room, they were removed from the racks individually, hung on the spindle of the sprayer, and sprayed during six revolutions. After this exposure the spray had begun to run off the fruit, thus approximating orchard treatment. The apples were then replaced on the racks and left in the warm laboratory until the spray had dried.

Subsequent treatment depended on the object of the particular experiment. When only the immediate toxicity of the sprays was being studied, codling moth eggs were placed upon the sprayed fruits as soon as they were dry and the racks put in the incubator. If the resistance of the spray deposit to weathering was to be investigated, the apples were held in a cool basement until the following morning, when one-half of the racks receiving each treatment were passed three times through the washer, being allowed to dry after each passage. A fan was sometimes used to hasten drying. As soon as they had dried after the final wash, eggs were put on both the washed and unwashed lots.

Infestation and Examination of Fruits

A very small piece of sticky tree-banding material was put on the point of the pin bearing the eggs to prevent entrance of the larvae beside the pin. The latter was then inserted into the upper side of the apple (but not through the wax) until the paper bearing the eggs was about one-sixteenth of an inch above the surface of the apple, care being taken to place the pin so that all larvae dropping from the paper would fall upon the fruit.

The racks of apples were immediately placed in an incubator at 77°F. and 85-90 per cent relative humidity, where they were held for three days. Forced circulation of air in the incubator was provided by a fan, running at a speed to produce a current perceptible to the hand at the outlet of the heating compartment. At slower fan speeds volatile compounds such as benzene hexachloride produced a misleadingly high mortality through fumigant action.

When the apples were removed from the incubator, the eggs were examined to determine the number which actually hatched; any larvae stuck in the sticky material on or about the pin were also recorded and subtracted from the total. An occasional larva extricated itself from the material and crawled a short distance before succumbing to its effects, but such larvae could be distinguished by their greasy appearance.

The apples were examined under a binocular microscope for larval entries, which were cut open to determine whether the larvae had become established. No attempt was made to follow larvae deeply into the fruit, because cannibalism was too prevalent to prevent the use of the actual number of living larvae as a basis for calculating establishment. Although unsuccessful entries ('stings') were recorded, they were generally too few and erratic to be of much significance, particularly on DDT-sprayed fruits.

Interpretation of Results

One or two lots of unsprayed apples were usually infested along with each day's series of spray tests, and the larval establishment in the check lots was used to calculate the efficiency of the spray treatments according to Abbot's formula.

There was always considerable day-to-day variation in the level of larval establishment allowed by any particular spray treatment, or even in unsprayed fruits, despite all attempts to eliminate variables in the method. This is often experienced in insecticide testing with other species but is probably more accentuated in the codling moth. Genetic constitution of the field-collected larvae, viability of the eggs produced by females ovipositing at different rates, and

progressive deterioration of the apples were among the factors which could not be held constant with the facilities at hand.

Valid comparisons could only be made among tests run on the same day. As only a limited number of trials could be conducted at once, any given series of tests was replicated in successive weeks. Although the degree of control might vary, the different materials usually maintained the same relative effectiveness in successive tests. A standard treatment of known efficiency, at first lead arsenate but later some particular DDT formulation, was usually included in each series as a basis for comparison.

Discussion

The technique which has been described was very valuable in screening the many possible formulations of DDT and other new insecticides so that only the more promising materials needed to be used in field trials for codling moth control. Most of the important conclusions derived from the laboratory tests have been confirmed by orchard experiments at Vineland Station or elsewhere.

For general insecticide-testing, especially where very precise comparisons were desired, the use of codling moth larvae in this method was not satisfactory. For example, an attempt at biological assay of the para-para content of different samples of technical DDT gave results which corresponded only roughly with the actual content as later determined by chemical analysis. In such investigations the test insect must be exposed to a measured amount of insecticide for a specific length of time, and the action of the insecticide must be restricted to one particular mode. It would be very difficult, if not impossible, to deposit a uniform and constant amount of insecticide over the variable surface of apples by spraying. Also, the insecticide may kill the codling moth larvae by contact or after ingestion, or apparently in some cases by fumigant action, and the larvae may escape further exposure if they succeed in penetrating the fruit.

The technique was also unsuitable for careful comparison of volatile insecticides such as benzene hexachloride because of variation in air circulation in different parts of the incubator.

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Nutritional Studies with *Blattella germanica* (L.) Reared Under Aseptic Conditions

I. Equipment and Technique[1]

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Introduction

In biological research it is often desirable to rear and handle animals free from microorganisms. This is particularly true in nutritional studies in which it is generally recognized that certain organisms in the digestive tract can supply nutritional factors which might otherwise be lacking in the diet. The possibility of this intervention makes it necessary to devise techniques for rearing and handling insects aseptically, in so far as the fauna and flora of their intestinal tract and of their environment are concerned. Consequently, a technique used throughout the present series of studies was developed making use of specially designed equipment by means of which nymphs of the cockroach, *Blattella germanica* (L.), were hatched and maintained under aseptic conditions, fed aseptic food, and kept aseptic throughout a series of measurements to evaluate their weight increment. The equipment and methods used constitute the basis of the present paper.

Cockroaches have been reared previously under aseptic conditions by other workers and the method they used provided a general basis for the present technique. These investigators developed a means of obtaining young nymphs free from microorganisms. Wollman (1926), sterilized the covering of the oothecum of *B. germanica* with mercuric chloride and reared the nymphs in sterilized flasks. However, very little detailed information is available on his technique. The work of Glaser (1930) and of Gier (1947) also shows that it is possible to free cockroach ootheca from external contamination by treatment with chemicals. It has been pointed out by Glaser (1930) that the nymphs developing within the oothecum are aseptic.

It is recognized that neither the earlier techniques nor the present one will eliminate intracellular symbionts. In developing the present technique for nutritional studies, the presence of intracellular symbionts, whose activities were reviewed by Steinhaus (1946), was not considered since the symbionts are transmitted from generation to generation, (Gier, 1936). They are always present in the cockroach and if they do actually exist as living organisms, they may be considered as having become physiologically part of the insect itself.

Description of Equipment

In the present study different equipment was designed and adapted to the aseptic handling of insects. The most important piece of apparatus is a glass cage in which the insect is reared individually. The cage is constructed from Kolle flasks and equipped with a food dish and watering device, as shown in Fig. 1. The entrance into the cage is plugged with an easily removable stopper made of non-absorbant cotton wrapped in gauze. The food dish is a shallow cup cut from the end of a 2 in. shell vial. The watering device consists of a 50 ml. Erlenmeyer flask, a tube through which water slowly passes from the flask and

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a similar tube through which air can be admitted. A hole is drilled through the back wall of the Kolle flask to receive the water tube and by this means sterile water may be passed into the cage. To construct the water tube an angle of approximately 90° is bent in a 4 mm. glass tube of a desired length. This tube, made with a slight constriction at its delivery end, is completed by inserting a glass wool wick throughout its length. The air vent is made from a piece of 4 mm. glass tubing bent at one end through approximately 140° and drawn to a very small diameter at the opposite end. A short length of glass tubing of about 5 mm. in diameter is packed with non-absorbent cotton to filter the air passing into the water bottle. These two parts are connected with a short piece of rubber tubing and a pinch clamp is applied to the connexion. The parts of the watering device are assembled on a two-hole rubber stopper and the entire assemblage is secured to the cage by means of a one-hole rubber stopper. The rearing cages are easily sterilized and are designed for use in combination with an aseptic transfer chamber.

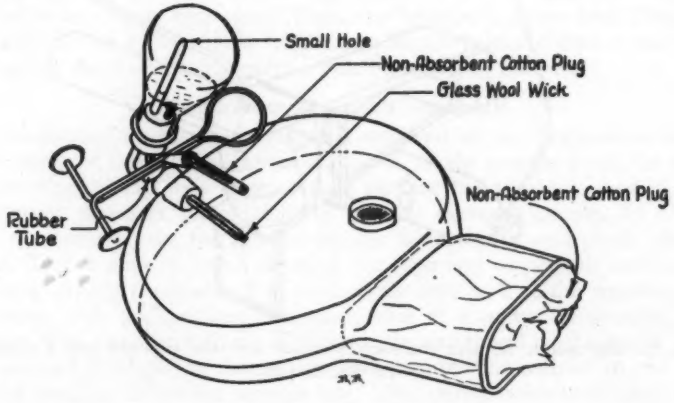

FIG. 1. Cage for aseptic rearing.

The transfer chamber is similar to that described by Reyniers (1943), but with certain modifications and the addition of special pieces of equipment, it was found to be more suitable for the present work. The chamber (Fig. 2) was constructed from ⅜ in. lumber. It is 36 in. long, 24 in. wide and 24 in. high with a glass window hinged to each side. The window frames are constructed to fit closely against the walls of the chamber. At one end of the chamber an air lock is constructed which includes a pair of sliding metal doors. By opening and closing these one after the other, equipment can be passed into the chamber without exposing the interior of the chamber directly to the outside. A pair of long rubber gloves inserted through each side of the chamber below the window are secured in place with the aid of three metal rings. The cuff of each glove, stretched over the inner ring, is clamped against the chamber when the two outer rings are bolted together. A small platform is located between the arm-holes to support a rearing cage and a hole is made through the side of the chamber to receive the neck of the cage while work is being done. The cage is pressed snugly against the opening and held securely in place by means of two small dowel sticks each of which is fastened through its middle to the edge of the platform. A sliding metal door covers the opening when the cage is removed. The chamber is painted inside and out with white enamel to obtain a smooth

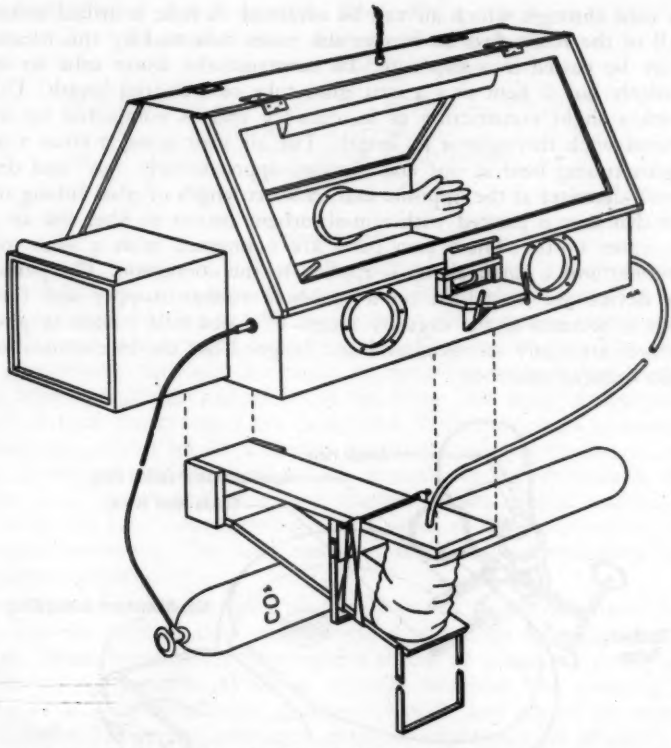

FIG. 2. The aseptic transfer chamber equipped with the aspirator and a supply of carbon dioxide.

easily cleaned surface. An ultra-violet germicidal lamp is installed along the top centre of the chamber to reduce contamination and assist in maintaining asepsis.

The chamber is equipped with special apparatus designed to facilitate handling living insects. The most useful piece of apparatus for this purpose is an instrument which was devised to catch and anaesthetize the insect with carbon dioxide. It consists of a long glass tube with a cup 25 mm. by 5 mm. in size formed at one end. The tube, serving also as the handle, contains loosely packed glass wool which picks up condensate. A coarse glass sinter at the junction of the handle and cup prevents the insect from entering the tube. Rubber tubing of sufficient length to reach all parts of the transfer chamber connects this glass appliance to a needle valve which is located inside the chamber. The valve, which is within reach of the hand, provides a means for regulating the flow of gas. Copper tubing fitted with suitable brass adapters connects the valve externally to the gas supply.

An aspirator was also built into the transfer chamber to assist in handling anaesthetized insects. It consists of a bellows (Fig. 2) with an air capacity of about 1½ liters installed below the transfer chamber where it can be operated with the foot by applying pressure on a metal stirrup. The movement of the bellows is restricted between a U-shaped metal guide and a wooden block. A downward movement of the foot on the stirrup causes the bellows to expand

and suck in air. When the foot is removed, the contraction of large rubber bands causes the bellows to collapse and forcibly expel the air. The rubber bands and the bag of the bellows were cut from the inner tube of an automobile tire. The bellows is connected to the transfer chamber by means of large rubber tubing, but inside the chamber a more flexible tubing of smaller diameter is used to conduct the air to a specially designed nozzle. This nozzle is made of 8 mm. glass tubing approximately 14 in. long, with a slight curve at the distal end. Near its proximal end a coarse glass sinter prevents the insect from being sucked back into the bellows. No air-borne microorganisms are introduced by the use of this aspirator since only the sterilized air of the transfer chamber passes through the bellows.

Although not an attached part of the transfer chamber, quartz fibre balances of the Salvioni type fulfil an important part in the work performed within the chamber. A series of three quartz fibres giving a range for weights up to 100 mgm. are mounted on an aluminum frame constructed in the form of steps. This frame is fastened with adjustable levelling screws to the end of an arm which can be rotated from a large ring stand. Thus, the balances occupy little floor space, can be adjusted to a convenient height or position in the chamber and can be moved out of the way during certain manipulations of the insect.

Preparation of aseptic materials

In conducting studies under aseptic conditions all microorganisms likely to interfere with the experiments must be avoided. In the present work the customary bacteriological methods were used to sterilize the apparatus, and thereafter precautionary measures were taken to prevent recontamination. In order to prepare the rearing cage for autoclaving the water bottle and pinch clamp are removed. This is done to avoid flooding the cage and to prevent corroding the pinch clamp during treatment. The exposed air inlet tube of the watering device was covered with a paper bag for autoclaving as a means of avoiding recontamination until the water bottle is replaced. It was found that a length of glass tubing inserted through the cotton stopper permitted the interior of the cage to dry more readily following autoclaving. The partly assembled cage and its water bottle, half-filled with distilled water, are autoclaved separately for 15 minutes at 15 pounds pressure. After removal from the autoclave each cage is placed in an oven and heated to a temperature of 105°C to remove all moisture. When the cage is dry the glass tube is withdrawn from the cotton stopper and the water bottle and pinch clamp are carefully replaced. The neck of the cage is inserted into a small paper bag in order to keep it free from dust while in use. After the rearing cage has been autoclaved it is opened only into the sterile transfer chamber in order to maintain asepsis.

Before using the transfer chamber its interior is thoroughly washed with disinfectant and it is exposed to ultra-violet radiation to eliminate microorganisms. The parts of the aspirator and of the anaesthetizing device used within the chamber are sterilized before installation. All equipment and instruments necessary for the handling of food and insects are sterilized before being passed into the chamber.

For the purpose of conducting nutritional studies with *B. germanica*, a number of experimental diets were used. These diets are dry granular solids consisting of amino acids, sterols, vitamins, minerals, and dextrin. They must be carefully prepared and sterilized for the feeding tests. To do this, each diet is placed in a large glass jar with a capacity of at least four times the volume of the food and the original screw cap is replaced with a paper covering. The large capacity of the jars makes it possible to spread the food in a thin layer when

the jars are turned on their sides for autoclaving. Thus the steam can thoroughly penetrate the food. It is autoclaved for 20 minutes at 5 pounds pressure. The steam is released rapidly from the autoclave in order to remove excess moisture. Drying is completed by placing the food in an oven at a temperature of 105°C. The jars of food are transferred to the aseptic chamber where the paper covers are removed. If the food has become caked it is broken down into its original granular form. The screw caps which were autoclaved separately are now replaced, and the bottles are stored at a low temperature until needed.

Aseptic nymphs are obtained by treating ootheca according to the technique described by Gier (1947). Only mature ootheca showing considerable pigmentation are removed from the female for treatment. Small test tubes containing slips of moistened blotting paper, plugged with cotton and autoclaved, serve as sterile hatching cages. Each treated oothecum is carefully inserted into a sterilized tube where the nymphs will emerge within a few hours. It became apparent, however, that the covering of the oothecum is affected by the treatment resulting in a decrease in nymphal emergence. Microscopic examination showed that the nymphs were alive, but were unable to rupture the capsule. A modification of the technique was introduced when it was found that the imprisoned nymphs can be liberated from the oothecum with the aid of forceps in the transfer chamber. Nymphs released in this manner appear vigorous and normal in every respect.

In aseptic nutritional studies only the food and the insects shown to be free from microorganisms are used. In order to detect the presence of microorganisms, samples of the autoclaved diets and of the nymphs obtained from treated ootheca are tested by customary bacteriological methods. In practice a few nymphs are reserved from each egg capsule for bacteriological tests. These nymphs and the remains of their egg capsule are macerated in a small test tube containing sterile water. Samples of each sterilized diet are prepared in a similar manner. Suspensions of the food and of the crushed nymphs are used as inoculum and are cultured by means of both surface and deep stab inoculations on proteose tryptone agar, nutritive caseinate agar and potato dextrose agar (anonymous, 1939). These are incubated at a temperature of 35°C for 72 hours. Any food found to be non-sterile is autoclaved again before offering it to the test insects. If it is shown that the nymphs are not bacteriologically sterile, all the cages which received a nymph from the contaminated oothecum are removed from the experiment. At the completion of an experiment similar bacteriological tests are made in order to make certain that aseptic conditions have been maintained. An insect is considered unsuitable for these studies and its growth data is omitted in evaluating a diet if the final bacteriological tests reveal that it has been reared non-aseptically.

Aseptic rearing and manipulations

During an experiment the transfer chamber and rearing cages as well as the insects are kept in an incubator room in which the temperature and the humidity can be controlled. The air in the room is circulated by means of an electric fan in order to equalize these environmental conditions throughout. The insects are reared in the dark, but a fluorescent lamp shielded to direct light into the transfer chamber is used while work is being done. The transfer chamber is placed in a convenient location at a suitable height on a table. The rearing cages are arranged on tiers of shelves and each cage is set in an inclined position by placing its neck on a strip of wood. In this position the high side of the cage will remain dry to provide the insect with some protection against drowning if the floor of the cage becomes flooded through faulty performance of the watering device. Al-

though the watering device was found to function quite satisfactorily, the arrangement of its parts causes it to be very sensitive to sudden changes in temperature. Consequently, it is considered advisable to keep the cages at the rearing temperature for several hours in order to test and properly adjust the watering devices before the insects are introduced.

The sterile rearing cages are opened under aseptic conditions with the aid of the transfer chamber. To do this, the small paper bag which protects the neck of the cage is removed and the neck is wiped with a strong disinfectant. The cage is fastened quickly upon the small platform of the transfer chamber with its neck inserted through the opening. Working within the attached rubber gloves, the cotton plug is removed from the cage and a number of manipulations may then be performed. Among these are replenishing the food supply with the aid of forceps and a glass spoon, stocking the cages with insects and weighing them at regular intervals throughout their growth period.

A simple routine has been devised to handle the insects aseptically. The test insects are reared individually to facilitate handling them and to avoid cannabalism, as well as to obtain growth data in a more readily analysable form. In order to stock each cage with one insect, the hatching tubes containing newly emerged nymphs are put into the transfer chamber and the nymphs are anaesthetized with carbon dioxide. Using the anaesthetizing instrument just enough gas is applied to immobilize the nymphs and yet permit their recovery in two or three minutes. For greater convenience in handling, the nymphs are put into a medium size glass weighing bottle provided with an easily removable lid. From this they can be transferred one at a time to the rearing cages with the aid of the aspirator. It was noted that small nymphs have considerable difficulty in turning themselves upright if placed on their backs in the cage. This may result in considerable mortality unless care is taken to place each nymph on its feet. When the various manipulations are completed the cotton plug is inserted into the cage with the aid of forceps, the cage is withdrawn from the chamber and the paper bag is replaced over the neck of the cage.

Each aseptic nymph is removed from its cage at regular intervals and is weighed on a Salvioni balance. The cage is opened in the usual way. The anaesthetizing instrument is inserted into the cage and carbon dioxide is directed around the nymph with the flow of gas reduced to the desired rate by means of the needle valve. Usually the nymph can be manoeuvred to drop directly into the cup-shaped end of the instrument and is removed with ease from the cage. If this cannot be accomplished, the anaesthetized nymph may be sucked into the aspirator and placed into the cup. The nymph is rolled from the cup directly into the weighing pan of the micro-balance assisted by the use of a camel's-hair brush. Before this is done, however, the weighing pan is removed and placed on the floor of the balance in order to avoid breaking the quartz fibres. Small forceps are used to handle the weighing pan. When the weight of the insect is determined it is returned to its cage by means of the anaesthetizing instrument or the aspirator. At this time it is convenient to replenish the food supply and make any necessary adjustments to the watering device. The cage is closed in the usual manner and is withdrawn from the transfer chamber. It is returned to the shelf when the nymph has recovered from the effects of the carbon dioxide and is again active on its feet.

Discussion

Through the use of the equipment described above a useful means has been developed for the rearing and handling of insects free from microorganisms in biological research. Previously insects have been reared aseptically in a manner

designed only to avoid the intervention of extracellular organisms. In the present work it has been possible to manipulate the insect aseptically during an entire experiment. Hence, the rate of growth can be determined by accurate measurements at regular intervals without either removing any individuals from their aseptic environment, or the necessity of depending solely upon observations and measurements made at the end of an experiment.

In the present series of studies over 300 nymphs were reared individually on sterile purified diets. Each nymph was removed from its cage and weighed weekly for twelve weeks in order to evaluate growth by determining its weight increment. Bacteriological tests have shown that less than one per cent of the treated nymphs handled in this manner became recontaminated with extracellular microorganisms. Thus, a means of avoiding the intervention of certain forms of microorganisms in the digestive tract of the insect has been provided and hence the response of an insect can be determined when these are absent. The technique provides an aseptic assay of the effects of diets upon growth over the entire developmental period.

In these studies twenty or more insects were handled per hour with considerable ease. This was made possible largely by the use of the anaesthetizing instrument and the aspirator to handle the insect. Injury to the insects is avoided since it is not necessary to handle them with forceps, although they are used in the transfer chamber to handle food dishes, the weighing pans of the balance and to assist in removing and replacing the cotton stoppers of the cages.

The habits of *B. germanica*, made it an ideal subject for nutritional studies. For use in feeding experiments this species possesses certain very desirable qualities. Among these are its size, incomplete metamorphosis and omnivorous feeding habits. Besides, it is easy to rear in the laboratory throughout the year. It multiplies rapidly and is easily handled. Although the present series of studies has been developed using the cockroach as test animals it is believed that the equipment and technique can be adapted with few modifications for similar work using other species.

Summary

Equipment and methods are described whereby nymphs of *B. germanica* were reared through their entire life cycle in an aseptic environment. A description is given of the equipment used for feeding, anaesthetizing and manipulating the transfer of nymphs for weighing. During the growth period of the insects provision was made to evaluate the effects of nutritional factors by weighing each individual at frequent intervals. The effectiveness of the technique is indicated by a contamination factor of less than one per cent in 300 trials.

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Spiders of the Nueltin Lake Expedition, Keewatin, 1947

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The spiders considered here were collected by Dr. Francis Harper during the period from May 31 to December 4, 1947, in the vicinity of Nueltin Lake, Keewatin, Canada while on a trip made under the auspices of the Arctic Institute with support from the Office of Naval Research.

A general account of the area and field notes have been given by Harper (1948, pp. 153-184) and Henry W. Fowler has presented a taxonomic report on the fishes (1948, pp. 141-152).

Eleven species of spiders belonging to five families are represented by mature specimens; and juveniles, not determinable to species, add another family.

LINYPHIIDAE

Estrandia nearctica (Banks) Figs. 1-3

One adult male, northwest corner of Nueltin lake, July.

Linyphia tridens Schenkel, from Kamtchatka, is apparently a synonym.

Lepthyphantes calcarata (Emerton)

One adult male, Windy river, Simon's lake, Oct. 20.

ARANEIDAE

Araneus patagiatus Clerck

One adult female, northwest corner of Nueltin lake, mouth of Windy river, Aug. 30.

LYCOSIDAE

Pardosa concinna (Thorell) Fig. 9

One adult female, Nueltin lake, Josie's bay, July 17. From open moor.

One adult female, northwest corner of Nueltin lake, mouth of Windy river, June 29.

Pardosa harperi n.sp

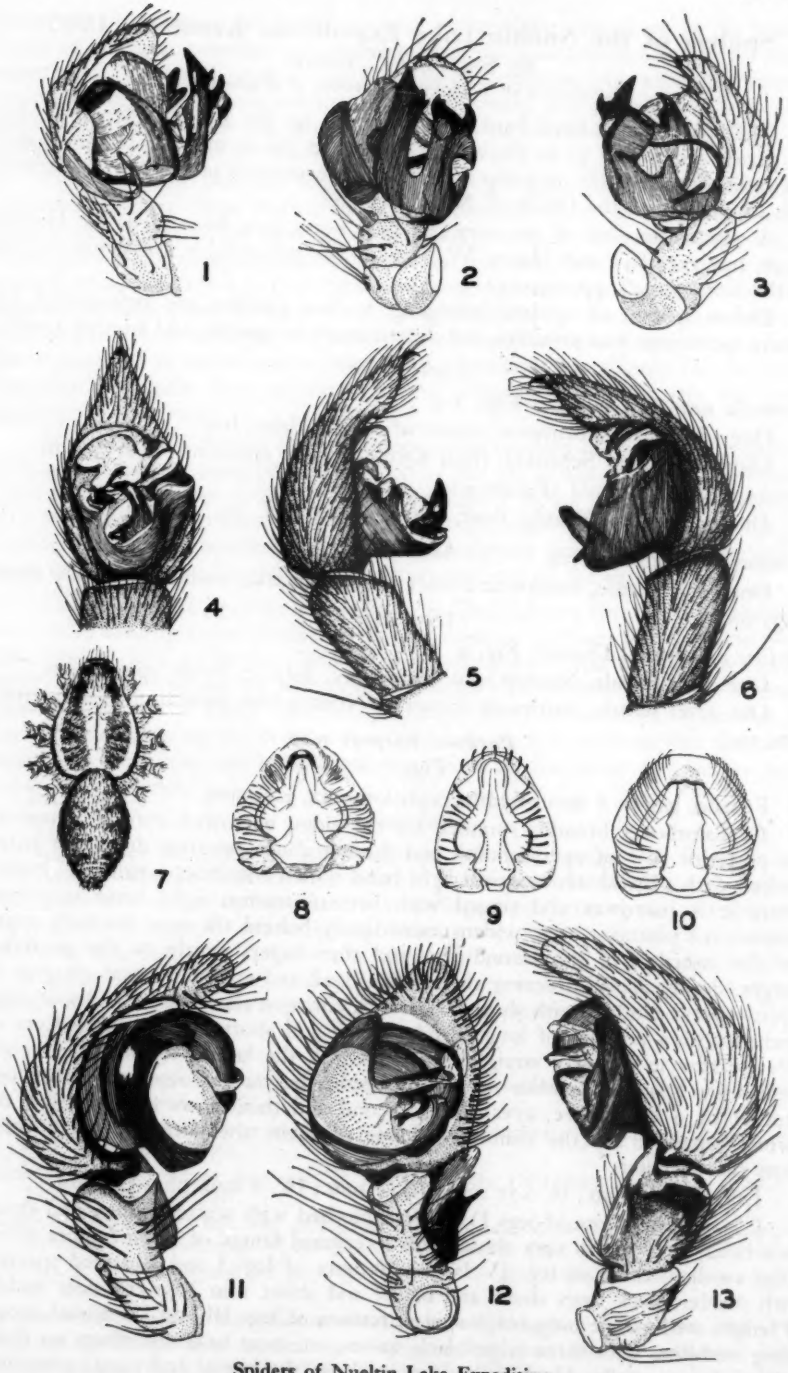
Figs. 7-8

Female, length 6 mm.; width, cephalothorax, 2.25 mm.

Cephalothorax broadly rounded on the sides, narrowed abruptly opposite the posterior row of eyes; smooth and shining above on areas devoid of hairs; marked with a broad sub-marginal light band which continues across the clypeus where it is narrower and tinged with brown; median light band originates between the posterior eyes, widens immediately behind the eyes, narrows opposite the insertion of the second legs and then tapers gently to the posterior margin; darker areas bordering the median band and extreme lateral margins of cephalothorax clothed with short, dark hairs; entire ocular area and sides of head clothed with a mixture of long, slender black and shorter white hairs. Eyes of anterior row in a nearly straight line, subequal, the lateral separated from the median by about the radius of one of them, the median separated from one another by a little more; eyes of the second and third rows equal in size, the former separated by the diameter of one of them, the latter by about three times the diameter.

Legs, I, 7.25 mm.; II, 6.75 mm.; III, 7 mm.; IV, 8 mm. plus, tips lost.

Coxae stout, those of legs IV largest, clothed with scattered long and short, dark hairs; trochanters very short, with a terminal fringe of hairs; femora somewhat swollen, those of legs IV largest; femora of legs I and II armed sparsely with slender black hairs above and below and above near base and near middle of length with single long black spines; femora of legs III and IV armed above, along mid-line, with three large black spines, one near base the others on distal half, and two smaller black spines, one each on the lateral and mesal sides near



Spiders of Nueltin Lake Expedition

tip; patellae narrower than femora and about one half as long, armed with two or three lateral and dorsal spines; tibiae slender, about as long as femora and armed below with three pairs of spines, the distal pair smallest; metatarsi a little shorter than tibiae, armed below with three pairs of spines; tarsi a little shorter than metatarsi, armed with short and long slender hairs.

Palpus slender, femur widened distally and armed above with three spines near distal margin, one near middle of length and one near base; patella short, with two spines above near base and one distally; tibia a little longer than patella, armed dorsally near middle of length with a single spine and on basal third, mesally, with two spines; tarsus a little longer than tibia, armed with three spines on basal third, above and mesally, and one spine mesally at mid-length. Chelicerae yellow and brown mottled, lower margin of the furrow with three small teeth; sternum yellowish-brown with darker mottlings.

Abdomen dark brown on the sides, dorsally with a broad median, dull yellow, band which is marked with faint, paired dark dots along the sides; abdomen thickly clothed with short, dark hairs and on the sides, anteriorly, with longer, silvery-white hairs; venter dull yellowish-white.

Epigynum with the median guide narrow anteriorly, broken each side at about mid-length, widely flaring behind; a broad and deep fovea on each side of guide becomes narrow and shallow anteriorly.

Holotype female.

Type locality Keewatin, northwest corner of Nueltin lake, mouth of Windy river, June 29, 1947, F. Harper collector.

This species is obviously related to *Pardosa concinna* (Thorell) (Fig. 9) and *Pardosa lapponica* (Thorell) (Fig. 10), as indicated by the character of the epigynum.

Pardosa uintana Gertsch Figs. 4-6.

One adult male, northwest corner of Nueltin lake, mouth of Windy river, July 7.

Pardosa xerampelina (Keyserling)

Two adult males, northwest corner of Nueltin lake, mouth of Windy river, July 7.

Tarentula asivik (Emerton)

One adult female, northwest corner of Nueltin lake, mouth of Windy river, July 22.

The epigynum bears little resemblance to the figure given by Emerton (1919, pl. 2, fig. 14) but Dr. W. J. Gertsch of the American Museum compared my specimen with some identified by Emerton and found the epigynum to vary considerably in different individuals. There is still the possibility that the Nueltin lake specimen may represent an undescribed species.

Tarentula pictilis (Emerton)

One adult male, Nueltin lake, near entrance of Josie's bay, July 16. Taken on a rocky hill in the barrens.

One adult male, northwest corner of Nueltin lake, mouth of Windy river, June 23.

GNAPHOSIDAE

Gnaphosa muscorum (L. Koch)

One adult male, northwest corner of Nueltin lake, mouth of Windy river, July 26; one adult male, same locality, July 30; one adult female, same locality, Sept. 29.

DICTYNIDAE

"*Hesperauximus*" *agelenoides* (Emerton) Figs. 11-13

One adult male, northwest corner of Nueltin lake, mouth of Windy river, Sept. 26.

Emerton (1919, p. 106) placed this species in *Amaurobius* from which it so obviously differs that Chamberlin (1947, p. 18) transferred it to the genus *Hesperauximus* Gertsch. But *agelenoides* also differs markedly from *Hesperauximus sternitzkii*, the type of the genus, and its final disposition must await the revisional work now being undertaken by Dr. Gertsch and Professor R. V. Chamberlin.

THOMISIDAE

Two juvenile specimens which possibly represent the genus *Misumenops*.

I am indebted to Dr. Gertsch for specimens of *Hesperauximus sternitzkii* and *Estrandia nearctica* and for comparing several of my specimens with northern spiders in the collections of the American Museum.

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Explanation of Figures

Fig. 1, *Estrandia nearctica* (Bks.), right palpus, lateral view; Fig. 2, same, ventral view; Fig. 3, same, mesal view; Fig. 4, *Pardosa uintana* (Gertsch), right palpus, ventral view; Fig. 5, same, lateral view; Fig. 6, same, mesal view; Fig. 7, *Pardosa harperi* n. sp., female from above; Fig. 8, same, epigynum; Fig. 9, *Pardosa concinna* (Thorell), epigynum; Fig. 10, *Pardosa lapponica* (Thorell), epigynum of female from Torne, Sweden; Fig. 11, "*Hesperauximus*" *agelenoides* (Emerton), right palpus, lateral view; Fig. 12, same, ventral view; Fig. 13, same, mesal view.